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Patients with Nonalcoholic Fatty Liver Disease Have a Low Response Rate to Vitamin D Supplementation

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Abstract

Background: Hypovitaminosis D is associated with an increased severity of nonalcoholic fatty liver disease (NAFLD), but reports on the response to cholecalciferol (vitamin D_3) supplementation are conflicting.

Objective: The objective of this study was to determine if standard vitamin D_3 supplementation is effective in NAFLD with hypovitaminosis D.

Methods: Sixty-five well-characterized adults [age (mean ± SD): 51.6 ± 12.3 y] with biopsy-proven NAFLD were screened. Forty-two patients (the ratio of men to women was 13:29) had hypovitaminosis D (plasma 25-hydroxyvitamin D [25(OH)D] <30 ng/mL). An observational study was performed in NAFLD patients with hypovitaminosis D treated with 2000 IU cholecalciferol (vitamin D_3) daily for 6 mo per clinical practice. Plasma 25(OH)D, hepatic and metabolic panels, and metabolic syndrome components were assessed before and after cholecalciferol supplementation. Body composition was measured by using bioelectrical impedance analysis. The primary outcome measure was plasma 25(OH)D ≥30 ng/mL at the end of the study. Secondary outcomes included change in serum transaminases, fasting plasma glucose, and insulin and homeostasis model assessment of insulin resistance (HOMA-IR). Chi-square, Student's *t* tests, correlation coefficient, and multivariate analysis were performed.

Results: Twenty-six (61.9%) patients had nonalcoholic steatohepatitis (NASH), and 16 (38.1%) had hepatic steatosis. After 6 mo of cholecalciferol supplementation, plasma 25(OH)D ≥30 ng/mL was observed in 16 subjects (38.1%; responders) whereas the remaining 26 patients (61.9%) were nonresponders with plasma 25(OH)D <30 ng/mL. Significantly fewer ($P < 0.01$) patients with NASH were responders (4 of 26, 15.4%) than those with hepatic steatosis (12 of 16, 75%). Baseline fasting serum alanine aminotransferase, plasma glucose, and HOMA-IR were similar in the responders and nonresponders, but the NASH score on the liver biopsy was lower (16.5%) in the responders ($P < 0.001$). Nonresponders had a higher fat mass (10.5%) and lower fat-free mass (10.4%) than responders did. End-of-treatment alanine aminotransferase and HOMA-IR improved only in responders. The baseline HOMA-IR and histological NASH score were independent predictors of nonresponse to cholecalciferol supplementation.

Conclusions: Daily supplementation with 2000 IU cholecalciferol for 6 mo did not correct hypovitaminosis D in the majority of patients with NASH. Further studies are needed to determine if higher doses are effective. This trial was registered at clinicaltrials.gov as 13–00153. *J Nutr* 2017;147:1938–46.

Keywords: nonalcoholic steatohepatitis, hypovitaminosis D, supplementation, vitamin D response, cholecalciferol

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease with a prevalence of nearly 30% in the population (1, 2). The spectrum of NAFLD includes hepatic steatosis (HS) and nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis (3). Targeting insulin resistance, an important pathogenic mechanism that contributes to NAFLD (4), has been a major strategy to prevent progression and potentially reverse NAFLD. However, the adverse effects of

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Abbreviations used: ALT, alanine aminotransferase; CRN, clinical research network; HS, hepatic steatosis; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic steatohepatitis score; NASH, nonalcoholic steatohepatitis; 25(OH) D, 25-hydroxyvitamin D.

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insulin sensitizers have limited the use of pioglitazone despite histological benefit (4–6). Vitamin E supplementation has been reported to be of therapeutic benefit in NASH, but concerns about higher all-cause mortality, risk of prostate cancer, and cerebral bleeding have tempered enthusiasm (4, 7–9). Obeticholic acid shows promise, but long-term efficacy and safety remain unknown (10). Furthermore, these therapies do not target the metabolic syndrome and the cardiovascular risk factors that are frequently associated with and contribute to clinical outcomes in NAFLD (11, 12). Thus, despite the high clinical significance, there are currently no approved therapies for NAFLD. Lifestyle modifications are the only consistent recommendations for NAFLD (12). There is increasing interest in vitamin D supplementation because of the frequent association between hypovitaminosis D and severity of NAFLD (13–17). Additionally, low plasma vitamin D concentrations have been reported in patients with cardiovascular disease, insulin resistance, diabetes mellitus, and obesity, all of which are associated with the metabolic syndrome and NAFLD (18–20). Hypovitaminosis D in patients with NAFLD and the metabolic syndrome is believed to be the result of decreased bioavailability in obesity due to storage in the expanded adipose tissue mass (21, 22). However, low plasma vitamin D in NAFLD has been reported to be independent of BMI (kg/m^2) and fat mass (23).

Low plasma vitamin D has also been associated with an increased histological severity of NAFLD (15, 16). The anti-inflammatory and immune-modulatory properties of vitamin D provide plausible mechanisms by which vitamin D may affect disease progression and severity in NAFLD (17, 24). The antifibrotic role of vitamin D has been shown in vivo in a murine model through the inhibition of hepatic stellate cell proliferation (25). Although there is evidence showing a strong association between vitamin D deficiency and NAFLD, there are limited data showing a clinical benefit with vitamin D replacement in preclinical models and patients with NAFLD (17, 20, 25, 26). Treatment with vitamin D supplements over 2 mo improved fasting glucose concentrations, insulin resistance, and HOMA-IR in patients with type 2 diabetes (27), although others have reported that vitamin D supplementation is beneficial only in nondiabetic, insulin-resistant subjects with metabolic syndrome (26). Replacement of vitamin D with 50,000 IU every 14 d for 4 mo in NAFLD patients showed beneficial effects in serum malondialdehyde and high-sensitive C-reactive proteins but did not show improvement in the serum hepatic enzyme concentrations (17). However, whether vitamin D replacement consistently corrects the deficiency in NAFLD and if there is a differential response of transaminases and metabolic components in responders and nonresponders are currently unknown.

Because obesity alters the bioavailability of vitamin D with a potentially impaired response to vitamin D supplementation (21, 28), changes in plasma vitamin D concentration with supplementation has been variable in NAFLD (17, 29–35). The response of plasma transaminases, a surrogate for hepatic histological response, to vitamin D replacement has not been consistent (7, 17, 29–31, 33). Finally, there are no data comparing the response of other metabolic components in responders and nonresponders to vitamin D replacement. The efficacy of vitamin D replacement in NAFLD, therefore, remains uncertain. We report the first prospective longitudinal study to our knowledge in well characterized patients with NAFLD to determine if daily supplementation with 2000 IU cholecalciferol orally for 6 mo would normalize vitamin D concentrations in NAFLD and if there were differences in the plasma transaminase response and changes in metabolic components between responders and nonresponders to the vitamin D replacement.

Methods

We evaluated 65 consecutive outpatients with well-characterized, histologically proven NAFLD who were not taking any supplemental vitamins in our metabolic liver clinic as part of the NIH-funded NASH clinical research network (CRN) (36). Of these, 42 (65.6%) had hypovitaminosis D defined as fasting plasma 25-hydroxyvitamin D [25(OH)D] <30 ng/mL, satisfied the inclusion and exclusion criteria, and agreed to be enrolled in a prospective replacement study (**Figure 1**). All subjects were adult patients (≥18 y of age) with histologically proven NAFLD before the start of any therapies known to be beneficial for NAFLD (vitamin E, pentoxifylline, pioglitazone, and/or prescribed diet and exercise and weight-loss programs). Patients with recent acute illness, clinical evidence of malignancy, or renal disease and those with medications known to affect vitamin D metabolism were excluded. Patients with other contributory causes of liver disease, such as excessive alcohol consumption (>21 drinks/wk and >14 drinks/wk for men and women, respectively; 1 drink has 14 g ethanol), hepatotoxic drug history, viral hepatitis, hemochromatosis, autoimmune hepatitis, Wilson's disease, or alpha-1 antitrypsin disease were also excluded. Subjects underwent a detailed history, physical examination, and clinical and laboratory evaluations. Alcohol intake was quantified and use of other medications documented.

Height, weight, BMI, and body composition with the use of an RJL Quantum X tetrapolar bioelectrical impedance analyzer (RJL Industries) were quantified at study entry as previously described (16).

Laboratory tests included fasting plasma glucose, insulin, lipid panel, hepatic function panel, including serum amino transaminases, blood urea nitrogen, serum creatinine, and plasma 25(OH)D concentrations, were quantified in the clinical core laboratory in all subjects at inclusion (within 2 wk of liver biopsy) and after ≥6 mo of cholecalciferol supplementation. All the tests except insulin and vitamin D assays were performed on a Roche Cobas 8000 on a c702 platform (Roche Diagnostics). Plasma 25(OH)D was assayed by a direct competitive chemiluminescent assay with the use of a Diasorin Liason XL immunoassay system (Diasorin). Plasma insulin was quantified by a 2-site sandwich immunoassay by using direct chemiluminescent technology on a Siemens Advia Centaur XP instrument (Seimens Diagnostics).

Components of metabolic syndrome, including type 2 diabetes mellitus and systemic hypertension, were identified and documented. Insulin resistance was calculated by using the HOMA-IR index.

Hepatic histology. The diagnosis of NAFLD was confirmed in all patients by liver biopsy by using the NASH CRN scoring system to calculate the NASH score (NAS) as previously described (37). An experienced hepatopathologist (AK) masked to the patients' clinical details scored the biopsy using the NASH CRN criteria (37). Briefly, severity was scored for the degree of steatosis from 0 to 3, fibrosis from 0 to 4, inflammation from 0 to 3, and hepatocyte ballooning degeneration from 0 to 2. The diagnosis of NASH compared with no NASH was based on the pathologist's overall evaluation of the histology and not the total NAS.

Study design. An observational study was performed in NAFLD patients with hypovitaminosis D who were treated with a daily dose of 2000 IU cholecalciferol (vitamin D_3) for ≥6 mo per clinical practice at MetroHealth Medical Center. Compliance was monitored by regular telephone calls and patient recall during office visits. The Institutional Review Board in Metro Health Medical Center approved the study, and a written, informed consent was obtained from all subjects. The study was registered at clinicaltrials.gov as 13–00153.

Outcomes. The primary outcome was an end-of-supplementation plasma 25(OH)D concentration ≥30 ng/mL. Secondary outcomes included changes in serum transaminases (alanine aminotransferase

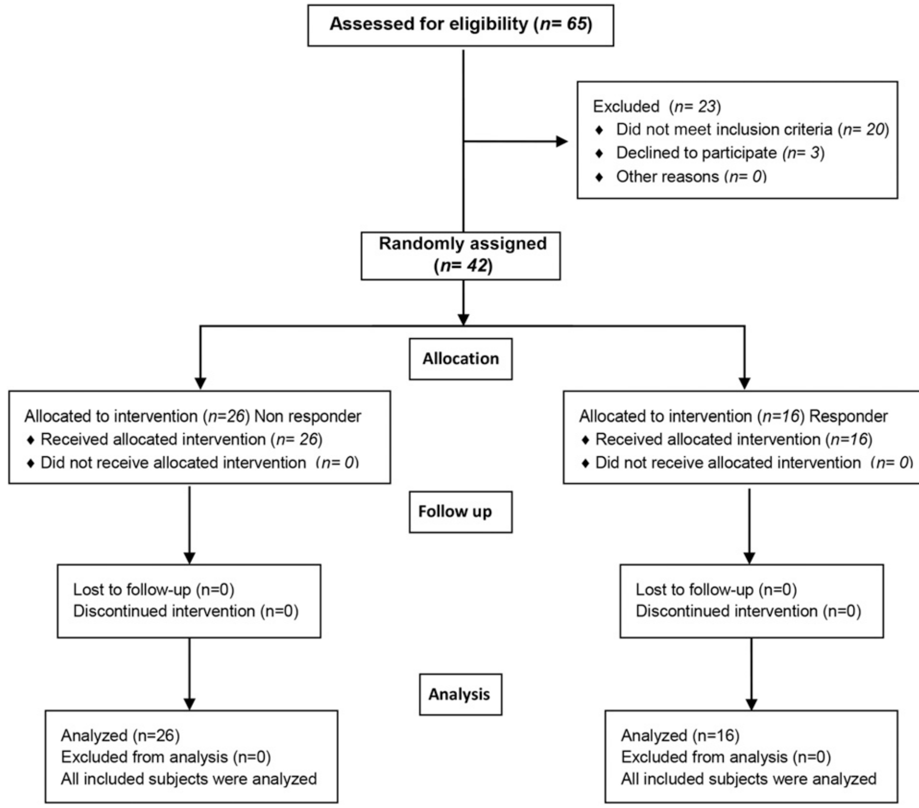

FIGURE 1 The Consolidated Standards of Reporting Trials flow diagram to determine the response to cholecalciferol (vitamin D_3) supplementation in patients with liver biopsy–proven nonalcoholic fatty liver disease.

[ALT] and aspartate aminotransferase), fasting plasma glucose, insulin, and HOMA-IR. Responders were defined as patients whose posttreatment plasma 25(OH)D concentration was ≥30 ng/mL, and nonresponders were those whose posttreatment plasma 25(OH)D concentration remained <30 ng/mL.

Statistical analysis. An intention-to-treat analysis was planned, but all subjects were documented as compliant with supplementation for ≥6 mo. All data are presented as means ± SDs unless specified. Qualitative variables were compared by using the chi-square test. Quantitative and rating variables were compared by using ANOVA with Bonferroni's correction or Student's *t* test for data that were normally distributed and the Kruskal-Wallis test or the Mann-Whitney *U* test for skewed data. For skewed data, nonparametric tests were used. Intention-to-treat analysis was used to determine if cholecalciferol supplementation reversed low plasma 25(OH)D as well as plasma transaminases. Response to therapy was evaluated by comparing the values before and after therapy by the paired Student's *t* test. The strength of association between quantitative variables was determined by Pearson's product movement correlation. Multiple regression analysis was performed to determine independent predictors of response to vitamin D supplementation. Statistical analyses were performed by using the SPSS v20.0 (IBM). The significance level was set at $P < 0.05$.

Results

The clinical and demographic characteristics of the patients with NAFLD are shown in **Tables 1** and **2**. All patients completed the study, no patients were hospitalized during the study, and there were no reports of any unusual symptoms. There were no significant differences in age or sex distribution between responders ($n = 26$) and nonresponders ($n = 16$). Obesity, diabetes mellitus, and hypertension were present in more than half of both the responder and nonresponder groups and were not significantly different. As shown in Table 1, the plasma 25(OH)D concentration at baseline was 21.7 ± 6.4 ng/mL in the entire cohort with significantly lower ($P < 0.01$) concentrations in the nonresponders than in the responders. The proportion of patients with obesity, diabetes mellitus, hypertension, and the use of statins,

TABLE 1 Baseline clinical features and body composition of patients with NAFLD who did or did not respond to supplemental vitamin D[1]

Characteristic	Nonresponders ($n = 26$)	Responders ($n = 16$)	Total ($n = 42$)
Age, y	50.2 ± 13.3	53.8 ± 10.5	51.6 ± 12.3
Sex, M:F	10:16	3:13	13:29
Obesity	21 (80.8)	11 (68.8)	33 (78.6)
Hypertension	15 (56)	10 (63)	30 (63)
Diabetes	14 (53.8)	8 (50)	22 (52.4)
NASH	22 (84.6)***	4 (25)	26 (61.9)
Plasma 25(OH)D, ng/mL	18.7 ± 5.0**	22.5 ± 4.6	21.7 ± 6.4
ACE inhibitor use	12 (44)	6 (38)	18 (42.8)
β-blocker use	4 (15)	4 (25)	8 (19)
Statin use	9 (38.5)	9 (56.3)	19 (45.2)
Height, cm	166.3 ± 12.7	161.9 ± 8.4	164.6 ± 11.3
Weight, kg	101.3 ± 23.60	92.98 ± 30.79	98.13 ± 26.52
Initial BMI, kg/m^2	36.5 ± 6.8	34.8 ± 8.7	35.9 ± 7.6
Fat mass, kg	59.5 ± 14.8**	35.29 ± 15.22	50.3 ± 19.0
Fat-free mass, kg	44.7 ± 13.3**	53.98 ± 12.47	48.2 ± 13.6
Fat mass, %	54.2 ± 10.1**	43.6 ± 11.59	46.8 ± 11.8
Fat-free mass, %	46.0 ± 10.0**	56.39 ± 11.6	53.25 ± 11.8

[1] Values are means ± SDs or *n* (%). Responders had a plasma 25(OH)D concentration ≥30 ng/mL and nonresponders had a plasma 25(OH)D concentration <30 ng/mL after 6 mo supplementation with 2000 IU oral cholecalciferol/d. *,**,***Different from responders: *$P < 0.05$, **$P \leq 0.01$, ***$P < 0.001$. ACE, angiotensin-converting enzyme; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; 25(OH)D, 25-hydroxyvitamin D.

TABLE 2 Baseline characteristics of patients with NAFLD with NASH or HS[1]

Characteristic	NASH (n = 26)	HS (n = 16)	P
Age, y	51.6 ± 13.0	51.6 ± 11.4	1.0
Sex, M:F	8:18	5:11	0.2
Obesity	21	11	0.3
Diabetes mellitus	16	6	0.5
Hypertension	15	10	0.5
Plasma 25(OH)D, ng/mL			
Baseline	19.3 ± 5.4	21.5 ± 4.5	0.2
End of treatment	33.9 ± 12.3	39.7 ± 7.6	0.001
Difference between baseline and end of treatment	3.9 ± 7.6	12.4 ± 11.8	0.007
Liver biopsy findings score			
Fat	2.0 ± 0.7	1.3 ± 0.8	0.001
Lobular inflammation	1.4 ± 0.5	0.9 ± 0.4	0.005
Hepatocyte ballooning	1.1 ± 0.7	0.3 ± 0.5	<0.001
Fibrosis	2.2 ± 1.2	1.3 ± 0.6	0.004
NAS	4.7 ± 1.2	2.6 ± 1.0	<0.001
Plasma TGs, mg/dL	162.7 ± 100.2	159.6 ± 95.4	0.9
Plasma HDL cholesterol, mg/dL	47.0 ± 20.2	40.4 ± 8.2	0.2
Plasma LDL cholesterol, mg/dL	117.9 ± 40.7	121.9 ± 29.6	0.7
Serum AST, IU/dL	52.5 ± 42.3	33.1 ± 17.2	0.04
Serum ALT, IU/dL	58.2 ± 26.0	43.6 ± 26.3	0.03
Plasma glucose, mg/dL	135.0 ± 65.5	101.9 ± 17.7	0.02
Fasting insulin, mIU/L	25.6 ± 14.6	17.5 ± 8.0	0.03
HOMA-IR score	7.6 ± 5.2	4.5 ± 2.2	0.01
BMI, kg/m^2	36.9 ± 7.2	34.2 ± 8.1	0.3
Fat mass, kg	59.47 ± 14.8	35.29 ± 15.2	<0.001
Fat-free mass, kg	44.7 ± 13.3	53.9 ± 12.5	0.03
Fat mass, %	51.9 ± 10.9	38.3 ± 7.7	<0.001
Fat-free mass, %	48.1 ± 10.9	61.7 ± 7.7	<0.001

[1] Values are means ± SDs or n. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HS, hepatic steatosis; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic steatohepatitis score; NASH, nonalcoholic steatohepatitis; 25(OH)D, 25-hydroxyvitamin D.

β-blockers and angiotensin inhibitors were similar among responders and nonresponders. The age, sex ratio, and proportion of patients with obesity, hypertension, and diabetes mellitus were similar in patients with NASH and HS (Table 2). There was no significant difference in plasma 25(OH)D concentrations in patients with NASH and HS at entry. Based on the end-of-supplementation plasma 25(OH)D concentrations, significantly fewer (P < 0.01) patients with NASH were responders (4 of 26, 15.4%) than those with HS (12 of 16, 75%).

Body-composition studies at baseline showed that there was no difference in BMI between responders and nonresponders (Table 1). However, nonresponders had greater fat mass (10.5% ± 3.4%) and lower fat-free mass (10.4% ± 3.4%) than did responders. The end-of-supplementation BMI was also similar (P > 0.1) in responders (35.0 ± 8.9) and nonresponders (36.7 ± 6.8). Consistently, there was no significant difference (P > 0.1) in the change in BMI between responders (0.18 ± 1.03) and nonresponders (0.17 ± 0.94). Patients with NASH had greater fat mass (19.1% ± 2.3%) than did those with HS (Table 2).

Laboratory values at inclusion in responders and nonresponders to cholecalciferol supplementation are shown in **Table 3**. Fasting serum transaminases, renal function, glucose, insulin, HOMA-IR score, and lipid profiles were not significantly different between nonresponders and responders to cholecalciferol supplementation. Hepatic histology showed greater NAS values and steatosis in nonresponders than in responders (**Table 4**). Histological evidence of liver injury and fibrosis was more severe in those with NASH than in those with HS even though all subjects had hypovitaminosis D (**Tables** 2 and 5). In the entire cohort of patients, initial plasma 25(OH)D concentrations were significantly inversely correlated with NAS (r = −0.312; P = 0.044),

TABLE 3 Baseline clinical chemistry of patients with NAFLD who did or did not respond to supplemental cholecalciferol[1]

Characteristic	Nonresponders (n = 26)	Responders (n = 16)	P
Plasma TGs, mg/dL	176.1 ± 107.4	137.9 ± 75.2	0.2
Plasma LDL cholesterol, mg/dL	123.4 ± 39.5	113.0 ± 31.2	0.4
Plasma HDL cholesterol, mg/dL	43.4 ± 11.0	46.3 ± 23.8	0.7
Plasma glucose, mg/dL	129.4 ± 62.4	109.5 ± 35.0	0.2
Plasma insulin, mIU/dL	20.7 ± 8.9	24.8 ± 17.5	0.4
HOMA-IR	6.04 ± 3.9	6.9 ± 5.6	0.6
Serum albumin, g/dL	4.1 ± 0.5	4.1 ± 0.3	0.9
Serum BUN, mg/dL	11.2 ± 5.4	14.4 ± 5.5	0.08
Serum creatinine, mg/dL	0.8 ± 0.2	0.9 ± 0.3	0.1
Serum total bilirubin, mg/dL	0.9 ± 0.5	0.8 ± 0.3	0.5
INR	1.0 ± 0.1	1.1 ± 0.5	0.8
Serum AST, IU/dL	43.5 ± 27.8	47.7 ± 47.3	0.8
Serum ALT, IU/dL	56.6 ± 35.5	46.2 ± 31.0	0.3

[1] Values are means ± SDs. Responders had a plasma 25(OH)D concentration ≥30 ng/mL and nonresponders had a plasma 25(OH)D concentration <30 ng/mL after 6 mo supplementation with 2000 IU oral cholecalciferol/d. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; INR, international normalized ratio; NAFLD, nonalcoholic fatty liver disease; 25(OH)D, 25-hydroxyvitamin D.

TABLE 4 Baseline hepatic histology in patients with NAFLD who did or did not respond to supplemental cholecalciferol[1]

Characteristic	Nonresponders score (n = 26)	Responders score (n = 16)	P
Steatosis	1.9 ± 0.8	1.4 ± 0.6	0.04
Inflammation	1.3 ± 0.6	1.1 ± 0.3	0.3
Ballooning	1.0 ± 0.8	0.6 ± 0.6	0.07
Fibrosis	1.9 ± 1.2	1.8 ± 1.1	0.8
NAS	4.1 ± 1.7	3.1 ± 1.1	0.02

[1] Values are means ± SDs. Responders had a plasma 25(OH)D concentration ≥30 ng/mL and nonresponders had a plasma 25(OH)D concentration <30 ng/mL after 6 mo supplementation with 2000 IU oral cholecalciferol/d. The range of scores are 0–3 for steatosis, 0–3 for inflammation, 0–2 for ballooning, 0–4 for fibrosis, and 0–8 for NAS. NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic steatohepatitis score; 25(OH)D, 25-hydroxyvitamin D.

and there was a nonsignificant inverse correlation with ballooning ($r = -0.276$; $P = 0.077$). Interestingly, there was no correlation between the initial 25(OH)D concentration and the degree of fibrosis ($r = -0.210$; $P = 0.18$). The changes in measured variables between the baseline and end-of-treatment values are shown in **Table 6**. At the end of therapy, the plasma 25(OH)D concentrations were normal in responders and remained subnormal in the nonresponders group, which was expected because the primary outcome was the normalization of plasma 25(OH)D concentration. The mean duration and mean dose of cholecalciferol supplement were similar in responders and nonresponders. Secondary outcomes, which included plasma ALT, showed a significant improvement ($P < 0.01$) at the end of treatment in the responder group compared with the nonresponder group (Table 6). Similarly, HOMA-IR scores showed improvement ($P = 0.02$) only in the responder group. Multivariable regression analysis showed that diagnosis (NASH or HS) and initial high HOMA-IR scores were independent predictors of nonresponse to cholecalciferol supplementation (**Table 7**). Interestingly, initial plasma 25(OH)D concentrations did not predict response to supplementation.

Discussion

We report for the first time to our knowledge that supplementation for 6 mo with the standard replacement dose is not sufficient to normalize plasma 25(OH)D concentrations in the majority of patients with NAFLD and hypovitaminosis D. We also observed that a greater proportion of NASH patients failed to respond to standard cholecalciferol supplementation compared with those with HS. Body composition studies showed that patients who were nonresponders had greater whole-body fat mass and lower fat-free mass. The response to cholecalciferol was associated with improvement in both hepatic transaminases and insulin sensitivity but did not affect the lipid abnormalities in NAFLD. Most, but not all, studies show that low plasma 1,25-dihydroxyvitamin D and 25(OH)D are associated with worsening severity of NAFLD (23, 38, 39). There are, however, conflicting data on the beneficial effects of vitamin D supplementation in NAFLD patients with hypovitaminosis D (17, 26, 27, 32, 33, 40, 41). Over the past decade, there has been increasing interest in vitamin D as a nutriceutical in the management of components of the metabolic syndrome (18). Our data provide the first prospective evidence that the current dosing regimen is ineffective in the majority of patients with NASH, and a longer duration and higher doses of vitamin D supplementation may be necessary to reverse hypovitaminosis D in NAFLD.

Observational studies have reported that low plasma vitamin D is associated with a worsening severity of NAFLD (16, 23, 38), even though a recent study and a systematic review question this association (42, 43). In addition to body composition and fat mass, plasma vitamin D concentration also depends on dietary or oral intake, absorption, plasma vitamin D–binding protein concentration, renal and hepatic metabolism, and expression of vitamin D receptor in cells (44). Because vitamin D is fat soluble, the volume of distribution would be expected to be greater in subjects with higher fat mass resulting in lower circulating concentrations as has been reported in the past (16, 21, 28, 45, 46). In the present study, there was an inverse correlation between plasma 25(OH)D concentrations and body fat mass. This was consistent with previous reports that hypovitaminosis frequently occurs in patients with NAFLD and obesity, both conditions associated with greater fat mass (16, 45, 46). This does not necessarily suggest a causal link between vitamin D concentration and whole-body fat mass but does support our contention that larger doses of vitamin D supplementation are necessary in NAFLD because the majority of these patients are obese. A recent report that loss of fat mass resulted in an increase in plasma vitamin D also supports this

TABLE 5 Clinical chemistry and NAS in patients with NAFLD who did or did not respond to supplemental cholecalciferol stratified into NASH and HS groups[1]

Characteristic	NASH			HS		
	Nonresponder (n = 22)	Responder (n = 4)	P	Nonresponder (n = 4)	Responder (n = 12)	P
Sex, M:F	8:14	0:4	0.3	2:2	3:9	0.6
Age, y	50.6 ± 13.2	56.8 ± 12.3	0.4	47.8 ± 15.7	52.8 ± 10.2	0.5
NAS	4.7 ± 1.3	4.5 ± 5.8	0.6	2.5 ± 1.7	2.6 ± 0.7	0.9
Change after 6 mo						
Plasma 25(OH)D, ng/mL	1.6 ± 5.4	16.3 ± 5.4	<0.001	0.4 ± 3.7	16.4 ± 10.8	0.001
Serum ALT, IU/dL	8.5 ± 13.5	−12.3 ± 14.7	0.01	42.3 ± 73.3	−9.4 ± 17.8	0.03
Serum AST, IU/dL	8.9 ± 8.3	−32 ± 42.9	<0.001	45.2 ± 89.2	5.3 ± 11.7	0.06
HOMA-IR	1.3 ± 4.2	−2.9 ± 4.6	0.08	9.6 ± 15.1	−1.2 ± 1.7	0.02
Plasma glucose, mg/dL	2.5 ± 69.9	−48.3 ± 87.3	0.2	33.3 ± 44.1	−4.0 ± 21.3	0.04
Plasma insulin, mIU/dL	5.5 ± 19.6	−14.1 ± 36.1	0.1	17.3 ± 21.4	−4.0 ± 6.3	0.006

[1] Values are means ± SDs. Responders had a plasma 25(OH)D concentration ≥30 ng/mL and nonresponders had a plasma 25(OH)D concentration <30 ng/mL after 6 mo supplementation with 2000 IU oral cholecalciferol/d. The change is over the 6-mo supplementation period. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HS, hepatic steatosis; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic steatohepatitis score (0–8); NASH, nonalcoholic steatohepatitis; 25(OH)D, 25-hydroxyvitamin D.

TABLE 6 Changes in plasma 25(OH)D and clinical chemistry in patients with NAFLD who did or did not respond to supplemental cholecalciferol[1]

Characteristic	Nonresponders (n = 26)	Responders (n = 16)	P
Plasma 25(OH)D, ng/mL			
Baseline	18.7 ± 5.0	22.5 ± 4.6	0.01
Final	20.1 ± 4.9	38.9 ± 7.8	<0.001
Change after 6 mo	1.4 ± 5.1	16.4 ± 9.5	<0.001
Duration of therapy, d	228.1 ± 81.3	197.6 ± 75.7	0.2
Mean total cholecalciferol intake, kIU/6 mo	456 ± 0.16	395 ± 0.15	0.2
Change after 6 mo			
Serum ALT, IU/dL	13.7 ± 30.9	−10.1 ± 16.6	0.002
Serum AST, IU/dL	14.5 ± 34.5	−11.9 ± 24.8	0.006
Serum bilirubin, mg/dL	0.0 ± 0.3	0.0 ± 0.4	0.1
Serum albumin, g/L	0.0 ± 0.3	0.1 ± 0.3	0.21
Plasma glucose, mg/dL	3.0 ± 66.4	−9.1 ± 49.0	0.06
Plasma insulin, mIU/dL	7.3 ± 20.0	−6.6 ± 17.6	0.7
HOMA-IR	2.5 ± 7.8	−1.7 ± 2.6	0.02

[1] Values are means ± SDs. Responders had a plasma 25(OH)D concentration ≥30 ng/mL and nonresponders had a plasma 25(OH)D concentration <30 ng/mL after 6 mo supplementation with 2000 IU oral cholecalciferol/d. ALT, alanine aminotransferase; AST, aspartate aminotransferase; NAFLD, nonalcoholic fatty liver disease; 25(OH)D, 25-hydroxyvitamin D.

contention (47). Our patient population included well-characterized patients with both NASH and HS. Despite previous reports that lower plasma vitamin D was associated with higher scores of individual components on liver histology in NAFLD (16, 38), we noted an inverse association only with the overall NAS. Scores of other histological components did not reach statistical significance. The lack of difference in individual components of the NAS may be related to either inclusion of only patients with low plasma vitamin D or to the fact that a large proportion of patients with NASH had a high NAS in our study. Despite the inverse correlation between plasma vitamin D concentration and NAS in this study, initial vitamin D concentrations were similar in patients with NASH and HS. One potential explanation is that we included only patients with NAFLD with hypovitaminosis D and did not include those with normal vitamin D concentrations. Another possible explanation is that low vitamin D is not associated with worsening histological severity of NAFLD as reported in a recent systematic review (43). However, unlike some individual studies that reported an inverse correlation between plasma 25(OH)D and histological scores of NAS components (16, 38), the systematic review dichotomized patients into those with a high NAS (≥5) compared with those with a low NAS (<5), which may explain the absence of significant differences in these 2 groups.

In addition to the number of studies that have related plasma vitamin D concentrations to hepatic histology, there have been 5 published randomized controlled trials and 1 observational study of vitamin D supplementation in NAFLD. These data are summarized in **Table 8**) (17, 29–33, 35). The results were heterogeneous in terms of the response of hepatic transaminases, lipid profile, and insulin resistance. The major limitation of these studies in adults with NAFLD treated with vitamin D is that the diagnosis, in all but one study in children (33), was not based on histology. Despite the rapid progress in our understanding of the natural history and pathogenesis of NAFLD, liver histology is still required to differentiate NASH from HS. We noted in our study that there was a greater likelihood of nonresponse in patients with NASH than in those without NASH. Of particular interest is the fact that in our subgroup of responders to vitamin D supplementation, hepatic transaminases and insulin resistance (HOMA-IR) also improved significantly. This was similar to that in other studies in which patients treated with vitamin D supplementation showed an

TABLE 7 Multivariate regression analysis of predictors of nonresponse to cholecalciferol supplementation in patients with NAFLD[1]

Model	Unstandardized coefficient, β	Unstandardized coefficient, SD	Standardized coefficient, β	t	P
Constant	0.302	0.530		0.570	0.6
Balloon	0.138	0.129	0.211	1.067	0.3
NAS	−0.084	0.077	−0.268	−1.088	0.3
NASH	0.712	0.216	0.721	3.297	0.002
Fat weight	0.000	0.003	0.026	0.112	0.9
Fat-free weight	0.000	0.003	−0.011	−0.061	1.0
Baseline HOMA-IR	0.042	0.015	0.391	2.813	0.008
Baseline serum ALT	00.000	0.002	0.013	0.100	0.9

[1] Dependent variable: responder and nonresponder groups. Responders had a plasma 25(OH)D concentration ≥30 ng/mL and nonresponders had a plasma 25(OH)D concentration <30 ng/mL after 6 mo supplementation with 2000 IU oral cholecalciferol/d. ALT, alanine aminotransferase; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic steatohepatitis score; NASH, nonalcoholic steatohepatitis; 25(OH)D, 25-hydroxyvitamin D.

TABLE 8 Published treatment trials for vitamin D supplementation in NAFLD[1]

Study (reference), year	Design and number of patients	Outcome measures	Conclusions
Della Corte et al. (33), 2016	41 children with liver biopsy before and 12 mo after the completed study; randomized, double-blind, placebo controlled; DHA + cholecalciferol 800 IU/d or placebo for 24 wk	BMI, hepatic transaminases, liver biopsy, HOMA-IR	The treatment group had a reduction in BMI, HOMA-IR, transaminases, and histology except fibrosis, similar to previous study with DHA alone. Cholecalciferol provided no additional benefit.
Papapostoli et al. (30), 2016	40 subjects; NAFLD based on transient elastography; cholecalciferol 20,000 IU/d for 7 d and then 20,000 IU/wk for 6 mo	Body composition by using BIA, hepatic transaminases, lipid profile, CAP score	The 25(OH)D concentration in serum normalized, and there was no change in body composition, LFT, or lipid profile–decreased hepatic fat accumulation.
Lorvand Amiri et al. (35), 2016	73 adults; NAFLD by ultrasound and clinical criteria; randomized, double-blind, placebo controlled; 500 kcal/d reduction ±1000 IU calcitriol/d for 12 wk	Body composition by using BIA, hepatic transaminases, blood glucose, lipid profile	There was a reduction in BMI and fat mass similar in both groups (calorie-restriction effect). The vitamin D–treated group had a greater reduction in transaminases and TGs, an increase in HDL cholesterol, and improvement in the ultrasound grade of hepatic steatosis.
Barchetta et al. (29), 2016	55 adults; diabetes, NAFLD by MRI and clinical criteria; randomized, double-blind, placebo controlled; 2000 IU cholecalciferol/d for 24 wk	MRI fat fraction, serum transaminases, CK18-M30, lipid profile, HOMA-IR, vascular variables	There was no improvement in any of the measured variables except plasma 25(OH)D concentrations, which increased in the treatment group.
Foroughi et al. (31, 32), 2014, 2016	60 patients; ultrasound and clinical diagnosis of NAFLD; randomized, placebo controlled; 50,000 IU cholecalciferol/wk for 10 wk	CRP, lipid profile, hepatic transaminases, BMI, HOMA IR, fasting glucose, insulin	There was no improvement in CRP, lipid profiles, hepatic transaminases, or BMI. HOMA-IR and plasma glucose decreased with cholecalciferol, but plasma insulin did not change.
Sharifi et al. (17), 2014	53 patients; ultrasound and clinical diagnosis of NAFLD; randomized, placebo controlled; 50,000 IU cholecalciferol every 2 wk for 4 mo	CRP, MDA, TNFα, TGFβ1, lipid profile, hepatic transaminases, sonographic liver fat, HOMA-IR	Serum 25(OH)D concentrations increased, and there was a reduction in MDA. No other variables improved.

[1] BIA, bioelectrical impedance analysis; CAP, controlled attenuation parameter; CK18-M30, cytokeratin 18 apoptosense M30 fragment; CRP, C-reactive protein; LFT, liver function tests; MDA, malondialdehyde; NAFLD, nonalcoholic fatty liver disease; 25(OH)D, 25-hydroxyvitamin D.

improvement in hepatic transaminases and lipid profile (33, 35) but differed from others who did not find a response to vitamin D supplementation (17, 29–32). None of the previous studies, however, stratified the responses based on improvement in plasma vitamin D concentration. Previous authors have reported that, despite a lack of improvement in lipid profile, insulin resistance, and serum transaminases, NAFLD patients treated with vitamin D supplementation had an improvement in circulating markers of oxidative stress (17).

The low response rate to supplementation in this study was in contrast to that in other studies in patients with NAFLD (17, 29–33, 35). One potential reason is that this is the first study to our knowledge in adults with histological diagnosis of NASH and HS, whereas the other studies used noninvasive measures (17, 29–32, 35). Low compliance could have been another possibility for the low response rates, but patients in the study were regularly followed in our metabolic clinic. Compliance was also monitored with telephone calls and in-person visits. Despite this, it is possible that inaccurate recall and reporting could have contributed to the low response rates. However, given that this was a once-daily regimen that has been reported to be more effective than intermittent therapy (48, 49), and regular evaluations in the metabolic clinic by a team with scheduled patient contacts in addition to easy access to the treating physician, noncompliance is an unlikely explanation. Lower vitamin D–binding protein may also contribute to the lower circulating vitamin D, and this cannot be excluded because this was not measured and is not a routine evaluation tool in most clinical settings (50). As mentioned earlier, a larger volume of distribution of vitamin D in the fat compartment is another potential reason for the lower response rates (21, 45–47, 51–53). Consistently, nonresponders had a higher whole-body fat mass than did responders, but on multivariate analysis fat mass did not reach statistical significance in predicting a response to supplementation. The response of plasma vitamin D concentrations to loss in fat mass also needs to be quantified, but any intervention that reduces fat mass is likely to have other beneficial effects, including improvement in hepatic function and potentially vitamin D metabolism. Other potential explanations, including impaired gut absorption, also need to be evaluated in future studies.

Another potential reason for the low response rate in the present study may be related to the dose of cholecalciferol used in this study (2000 IU/d). This is, however, an unlikely reason because we used >3 times the current recommended daily intake (600 IU) for 6 mo and yet found a low response rate of only 38% (51, 54). The validity of current guidelines and recommendations for vitamin D supplementation has been questioned (55–60). Whether the regimen should depend on the initial concentration, hepatic or renal function, body composition, etc. is currently not known. The recommended daily intake of vitamin D may also greatly underestimate the true daily needs in humans (54). There are also emerging data that show that vitamin D supplementation needs to be tailored to body weight and possibly whole-body fat mass (51). Based on published regression equations, for every 40 IU of dietary intake of vitamin D, plasma concentrations are expected to increase by 0.28 μg/L over a 5-mo period (55, 61). By using these estimates, for a supplementation of 2000 IU/d for 6 mo, we expected an increase of $\geq$14 μg plasma 25(OH)D/L. Instead, we observed a mean change of only 7.12 μg/L (range: -8.3 ± 35.7 μg/L) in this population. Based on these estimates, we predict that doubling the dose administered to 4000 IU/d for 6 mo may be necessary for correction of the hypovitaminosis D in NAFLD. Comparison of daily, weekly, or monthly dosing showed that daily dosing is the most effective and tolerated (62). Similarly, a cholecalciferol (vitamin D_3) supplement is believed to be better than vitamin D_2, and hence we used cholecalciferol for supplementation in this study (28). Even though there are suggestions that doses >2000 IU/d may be needed in patients with deficiency, the upper limit of the current dosing recommendation was 2000 IU/d. Because there is the potential for vitamin D toxicity, including hypercalcemia, the current recommended dose by the Endocrine Society was used in this study (55–58, 63).

This prospective, observational study of cholecalciferol supplementation in a select cohort of well-characterized NAFLD patients with vitamin D deficiency [plasma 25(OH)D <30 ng/mL] showed that the currently used regimen of 2000 IU/d for 6 mo is insufficient, and higher doses may be necessary in the majority of patients with NASH. Bioavailability and kinetic studies are necessary because normalizing plasma 25(OH)D has clear metabolic benefits with improvement in hepatic transaminases. Even though we did not have an end-of-treatment biopsy, previous studies have suggested that serial hepatic transaminases are a good indicator of treatment response, and a liver biopsy is not always necessary to demonstrate a response to an intervention (7). Our studies also lay the foundation for aggressive strategies to lower body fat mass to complement vitamin D supplementation to improve the clinical course of NAFLD.

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